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Decreased Omega-3 Long-Chain Polyunsaturated Fatty Acid and Total Omega-3 Polyunsaturated Fatty Acid Content of Chicken Meat Fed Diets High in Linoleic Acid to Alpha-Linolenic Acid Ratio

Research Article

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ABSTRACT

The aim of this study was to evaluate the effect of varying linoleic acid (LA) levels in diets on the conversion of alpha-linolenic acid (ALA) into eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) levels in chicken meat. The fat content of the feed was adjusted by adding pure or blended vegetable oil and the oils were included at a level of 2.8% in order to produce diets with the desired levels of LA and ALA. The ALA content in the diets was held constant at 2.1% energy (%en) while the level of LA varied from 2.9 to 4.4%en. The LA to ALA ratio of the experimental diets thus ranged from 1.4:1 to 2.1:1. The findings indicated that the total n-3 long-chain polyunsaturated fatty acids (LCPUFA) levels in the breast meat of chicken fed with the lowest LA content was 16% higher than the n-3 LCPUFA levels in the breast meat of those fed with the highest LA content. In general, the decrease in the level of n-3 LCPUFA due to inhibition by LA was less than the stimulatory effect of an equivalent level of ALA on n-3 LCPUFA accumulation. This study showed that the strongest influence on n-3 LCPUFA accumulation in chicken meat was the level of ALA in the diet. The experimental diets did not appear to influence the growth performance of chickens. In conclusion, there was only a modest impact of dietary LA on omega-3 LCPUFA accumulations in chicken meat, but diets that are lower in LA level will allow greater conversion of ALA into n-3 LCPUFA.

KEY WORDS alpha-linolenic acid, chicken tissues, diets, linoleic acid, omega-3 PUFA.

INTRODUCTION

The consumption of omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), provides beneficial health effects which has been well documented (Nguyen *et al*. 2018; Tocher *et al*. 2019). To increase the intake of n-3 LCPUFA, one approach that can be taken is to increase the intake of fish or fish processed products, which are known to be the main food source of n-3 LCPUFA. However, many people do not consume enough fish in their diet. In contrast, broiler chicken meat is a popular food and is eaten in large quantities. Increasing the levels of n-3 LCPUFA in such meat can be an alternative strategy to increase the consumption of n-3 LCPUFA without changing existing eating habits. Moreover, with components that have beneficial effects on human health, this strategy of food enrichment can produce functional

foods.

One strategy to increase the natural production of n-3 LCPUFA in chicken meat is by incorporating vegetable oils rich in n-3 PUFA, alpha-linolenic acid (ALA, 18:3n 3), in their diets (Kartikasari *et al*. 2012). There have been a number of reports of the n-3 fatty acid enrichment of chicken meat through dietary enrichment with vegetable oils containing the n-3 precursor, ALA. Vegetable oils such as chia seed (Ayerza, 2009), flaxseed, canola (Nguyen *et al*. 2018; Rodríguez *et al*. 2019), and purslane (Uddin *et al*. 2014) are rich sources of ALA. A number of studies have reported an increase in n-3 PUFA levels in chicken meat following the consumption of diets supplemented with vegetable oils, such as soybean, flaxseed and rapeseed oil, rich in ALA (Stanacev *et al*. 2014; Kalakuntla *et al*. 2017; El-Bahr *et al*. 2021). A study in laying hen found that increasing the amount of ALA in the diet up to a level of 6%en while keeping LA constant enhanced the levels of n-3 LCPUFA (EPA, DPA, and DHA) and total n-3 PUFA of eggs (Kartikasari *et al*. 2021). The use of ALA rich sources derived from seed oils in order to increase productive performance and n-3 PUFA content was also reported in ruminants (Nguyenn *et al*. 2018), and the studies showed that supplementing the diets of lamb with ALA-rich sources at or below 6% can promote n-3 PUFA profiles in lamb meat without resulting in any detrimental impact on growth, carcass and sensory properties. Moreover, a study conducted by Kartikasari *et al*. (2012) found that diets with high ALA content increased the content of EPA and DHA in chicken thigh meat to levels of 5 and 4-fold, respectively, relative to birds fed low ALA diets. The improvement in meat n-3 LCPUFA status was achieved by varying the ratio of LA to ALA by increasing the level of ALA in the diets and keeping a constant LA level in order to optimize the conversion of ALA into EPA and DHA. The levels of n-3 LCPUFA achieved in that study were higher than the levels reported in previous studies (Febel *et al*. 2008; Zelenka *et al*. 2008). However, there is competition between LA and ALA for the use of desaturation and elongation enzymes in fatty acid metabolism (Simopoulos, 2016). The conversion of the n-3 precursor, ALA, into n-3 LCPUFA, EPA and DHA is influenced by the availability of substrate and levels of desaturation and elongation enzymes. However, there is potential for competitive inhibition between n-6, linoleic acid (LA, 18:2n-6) and n-3 PUFA, ALA in the diet (Calder, 2016; Husted and Bouzinova, 2016). This could be because these enzymes are also involved in the conversion of LA to arachidonic acid (AA, 20:4n-6). Therefore, high dietary intake of LA has the potential to reduce the content of n-3 LCPUFA, EPA, DPA and DHA and favour a high conversion of LA to AA, which is done by inhibiting ALA access to key enzymes such as delta-6 desaturase (Gibson *et al*. 2013). Accordingly, the ratio of LA to ALA in the diet is a major determinant for the optimal conversion of ALA to n-3 LCPUFA. The results of studies conducted by Kartikasari *et al*. (2012) highlighted that the conversion of ALA was mainly driven by the level of ALA in the diet. The lowest LA to ALA ratio of experimental diets resulted in the highest EPA, DPA and DHA in these tissues. These results indicated that increasing the ALA content of the diets was important in the regulation of EPA, DPA and DHA accumulation in chicken meat.

However, a high consumption of LA can reduce the production of EPA and DHA and favour high AA (Liou *et al*. 2007). So, we conducted this current study to evaluate the impact of LA levels in diets on the conversion of ALA into n-3 LCPUFA, EPA, DPA, DHA, and total n-3 fats of chicken meat.

MATERIALS AND METHODS

Animal, experimental design, and rearing

This study was approved by the Animal Ethics Committees of the Department of Primary Industries and Resources South Australia and the University of Adelaide. The variable factor in the experimental diets was LA while the level of ALA was kept constant. There was a total of four dietary treatments, namely, a reference diet that corresponded to the control diet and three experimental diets. These diets were provided to a total of 64 one-day-old mixed chicks (Cobb 500) obtained from the Baiada hatchery (Willaston SA, Australia). The birds were randomly allocated to one of the four diets in eight pens with 8 birds/pen. Each dietary treatment was assigned to two pens. The chickens were housed for 28 days and reared at PPPI, SARDI, Roseworthy Campus under controlled environmental conditions.

The chickens were immediately weighed in groups of 8 (Libror EB-32KS SHIMADZU) upon arrival from the hatchery and placed on brown paper in rearing cages (1.2×0.9 m^2 each). Feed was given in a plastic hopper and also spread over paper to encourage the chickens to eat immediately. Fresh water was placed in the splash cup under the drinking nipples to encourage the chickens to drink immediately after being placed. During the research, both feed and water were provided *ad libitum*. The room temperature was maintained at 27 ˚C for 4 days and gradually lowered to 20 ˚C over the experimental period. All birds were subjected to a 24 hour lighting program during the growing period.

The cage was also heated using an infrared lamp (175 watts) for 21 days. The chickens were observed periodically during the first few days to ensure that they were comfortable with environmental conditions and that all had access to adequate feed and water.

The room temperature was maintained by a logic controller (Tempron 606) which regulates airflow, cooling and heating. Fresh wood shavings were placed on the floor of the cage at the age of 3 days and during the experimental period fresh shavings were added three times.

Dietary treatments

The three experimental diets were based on a standard commercial starter diet with a low level of fat. Ingredient composition and nutrient content of basal diet is presented in Table 1. The fat content of the feed was adjusted by adding pure or mixed vegetable oil. The mixed vegetable oils were obtained by varying the proportions of several vegetable oils including macadamia, flaxseed and sunflower oils. The pure or blended vegetable oils were included at a level of 2.8% in order to produce diets with the desired levels of LA and ALA. The dietary treatments had the same nutritional values as the basal diet except for the fatty acid composition of the fats. The LA level of reference or control diet was 2.3%en with low ALA level (0.2%en). The experimental diets were formulated by varying the levels of LA, which was 2.9 (T1, diet 1), 3.8 (T2, diet 2), and 4.4%en (T3, diet 3), with ALA levels kept constant at 2.1%en. These resulted in the ratio of LA to ALA varying from 1.4:1 (T1, diet 1) to 2.1:1 (T3, diet 3). The total fat content was kept constant at approximately 5%. The fatty acid composition of the experimental diets is shown in Table 2. Each dietary treatment was provided *ad libitum* for the duration of the 28-day growth period. All experimental diets met or exceeded the nutritional requirements recommended by the NRC (1994) for broiler chickens.

Sample collection analysis

At 28 days post-hatch, six selected birds from each pen (12 birds per group) were weighed individually and breast and thigh tissues were collected as described by Kartikasari *et al*. (2012). The total lipids in the diet and tissue samples were extracted following the method of Folch *et al*. (1957) and subsequently, the fatty acids were transmetylated by the procedure as described in previous studies (Tu *et al*. 2010; Kartikasari *et al*. 2012), using 1% H_2SO_4 in methanol at 70 ˚C for 3 hours. After cooling, the resulting fatty acid methyl ester (FAME) was extracted with n-heptane and then transferred to a gas chromatography (GC) vial containing anhydrous sodium sulfate (about 30 mg). The samples were then stored at 20 ˚C and the fatty acid composition of tissue samples was determined and quantified by GC.

Statistical analysis

The data of fatty acid profiles was analysed by One Way ANOVA with a completely randomised design using GenStat-tenth edition version 10.1.0.72. The variable factor in the experimental diets was LA levels while the level of ALA was kept constant. There was a total of four dietary treatments, namely, a reference diet that corresponded to the control diet, and three experimental diets, with 12 replicates per group (Table 2). Statistically significant results among treatments were further analysed using Tukey's test at the 95% confidence level ($P<0.05$).

RESULTS AND DISCUSSION

Fatty acid composition of liver, breast, and thigh phospholipids from chickens fed dietary treatments varying in LA content (LA to ALA ratio) for 28 day is shown in Tables 3, 4 and 5. Elevating the dietary concentration of LA as energy from 2.9 to 4.4%en (LA to ALA ratio from 1.37:1 to 2.06:1) whilst keeping a constant ALA level at 2.1%en had an effect ($P<0.05$) on ALA, EPA, and total n-3 fatty acid levels in liver phospholipids (Table 3). Increasing the LA content of dietary fatty acids to a level of 3.8%en LA caused a decrease in the level of ALA, EPA and total n-3 PUFA in liver tissue samples to 22.45, 31.83, and 26.61% of total fatty acids, respectively ($P<0.01$). There was no difference in liver ALA levels (Table 3) between diets containing 3.8%en LA (T2) and 4.4%en (T3). Similarly, the experimental diets caused a consistent reduction in EPA, DPA, DHA, and total n-3 fatty acid content in breast (Table 4) and thigh tissue (Table 5). The dietary treatment with LA content of 4.4%en (also the highest LA to ALA ratio) significantly decreased the proportion of breast tissue EPA ($P<0.01$) and total n-3 fatty acids ($P<0.05$) to 23.95 and 12.4%, respectively, lower than the diet with an LA level of 2.9% (the lowest LA to ALA ratio). The changes in n-3 fatty acids as a result of the dietary treatments were somewhat tissue specific. In the breast samples, there was no significant difference in the level of ALA and DHA by increasing dietary LA content whereas in thigh, the level of ALA and DHA responded to dietary LA ($P<0.05$). It appeared that the increased LA levels as energy from 2.9 to 4.4%en in the diets resulted in a lower DHA content ($P<0.05$) and significantly reduced total n-3 fats in thigh meat ($P<0.05$).

The decrease in n-3 LCPUFA (EPA, DPA, and DHA) levels in liver, breast, and thigh tissues might be due to competition between ALA and LA, the precursor to n-3 LCPUFA, for delta-6 desaturase. ALA and LA, the 18-carbon PUFA in food, are the dietary essential fatty acids. In the bodies of human and animals, both ALA and LA can be lengthened to 20- and 22-carbons, the LCPUFA (Simopoulos, 2016).

Since ALA and LA have homologous structures, they compete for the same desaturation and elongation enzymes (Calder, 2016).

Table 1 Ingredient composition and nutrient content of basal diet

Ingredients[1]	kg/100 kg
Wheat fine	43.91
Wheat mil vits	0.80
Barley	10.00
Triticale fine	10.00
Peas fine	10.00
Meat meal	4.60
Blood meal	1.40
Soybean meal	15.00
Millrun	2.00
Limestone small	0.79
Salt	0.18
Sodium bicarbonate	0.27
Choline chloride 75%	0.07
Potassium carbonate	0.01
L-threonine	0.09
Alimet	0.35
Standard broiler starter premix	0.20
Lysine sulphate	0.29
Phyzyme XP5000L broiler	0.01
Feed enzyme premix	0.03
Nutrient content (%)	
ME, kcal/kg	2787
Protein	22.99
Fat	2.20
Fibre	3.82
Calcium	0.98
Phosphorus	0.74
Available phosphorus	0.5
Na	0.2
K	0.71
Cl	0.2
Lysine	1.3
Methionine	0.59
Methionine + cystine	0.99

[1] A standard commercial starter diet (Ridley Agriproducts Pty Ltd, Murray Bridge, Australia).

Table 2 Fatty acid contents of the diets

Item	Experimental diets		
	Diet 1 (T1)	Diet 2 (T2)	Diet 3 (T3)
Linoleic acid (LA, % en)	2.90	3.78	4.38
Alpha-linolenic acid (ALA, % en)	2.12	2.06	2.13
LA:ALA ratio	1.37:1	1.83:1	2.06:1
Fat content (%)	5.11	5.18	5.15
Fatty acid (FA)			
Total saturated FA	19.41	18.53	17.97
Totals Trans	0.42	0.41	0.43
18:1n-9	27.11	23.64	19.94
18:1n-7	1.58	1.26	0.96
Total monounsaturated FA	33.80	27.86	21.93
Total n-9	28.34	24.59	20.63
Total n-7	5.36	3.20	1.25
18:2n-6 (LA)	26.52	34.09	39.78
Total n-6	26.76	34.33	40.04
18:3n-3 (ALA)	19.37	18.63	19.38
Total n-3	19.52	18.75	19.48
Total polyunsaturated FA	46.28	53.08	59.52

Table 3 Fatty acid profiles of liver phospholipids from chickens fed diets varying in linoleic acid (LA) to alpha-linolenic acid (ALA) ratio for 28 days[1]

Item	Experimental Diets				
	T1	T2	T3		
LA (% en)	2.90	3.78	4.38		
ALA (% en)	2.12	2.06	2.13		
LA:ALA ratio	1.37:1	1.83:1	2.06:1		
Fat content (%)	5.11	5.18	5.15		
Fatty acids	**(% of total fatty acids)**			**PSEM**	**Significance**
16:0	17.38	16.78	17.43	0.462	NS
18:0	24.79	25.23	24.75	0.483	NS
SFA	43.87	43.59	43.99	0.191	NS
18:1n-9	12.05	11.93	10.58	0.703	NS
18:1n-7	1.75	1.68	1.46	0.102	NS
MUFA	15.81	15.63	13.74	0.809	NS
20:3n-9	0.91	1.00	0.61	0.069	NS
18:2n-6 (LA)	17.62^{a}	20.35^{b}	20.80^{b}	0.36	*
20:3n-6	1.36	1.60	1.47	0.071	NS
20:4n-6 (AA)	7.55	7.80	8.81	0.620	NS
Total n-6	27.54^{a}	30.93^{b}	32.47^{c}	0.316	**
18:3n-3 (ALA)	0.98^{b}	0.76^{a}	0.89^{ab}	0.013	**
20:3n-3	0.17	0.14	0.18	0.015	NS
20:5n-3 (EPA)	2.89^{b}	1.97^{a}	1.95^{a}	0.090	**
22:5n-3 (DPA)	1.92	1.34	1.75	0.249	NS
22:6n-3 (DHA)	5.51	4.19	4.03	0.342	NS
Total n-3	11.50^{b}	8.44^{a}	8.82^{a}	0.537	*
Total PUFA	39.04	39.37	41.29	0.761	NS
	Fat content (% fresh weight)				
	5.06	5.43	4.81	0.593	NS

[1] Values are means of twelve observations (n=12) per treatment and their pooled standard error of the mean (PSEM).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid and PUFA: polyunsaturated fatty acid.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.

The competition between LA and ALA in the use of the same desaturase and elongase enzymes for the bioconversion of ALA to n-3 LCPUFA has been identified (Nain *et al*. 2012). Increasing the dietary ALA level might increase n-3 LCPUFA, EPA, DPA and DHA accumulation (Gibson *et al*. 2013). In contrast, high consumption of LA can reduce the production of EPA, DPA and DHA and favour high arachidonic acid, AA (Liou *et al*. 2007). Several studies have highlighted that by modifying the dietary balance of LA and ALA, the precursors of n-6 and n-3 LCPUFA, in the diet, the levels of n-6 and n-3 LCPUFA in animal tissues can be regulated (Tu *et al*. 2010; Kartikasari *et al*. 2012). A study showed that at appropriate dietary ALA levels, increased dietary LA levels resulted in a reduction in metabolites (Gibson *et al*. 2013). Thus, a high dietary LA to ALA ratio leads to a decrease in the metabolism of ALA to EPA, DPA and DHA. Komprda *et al*. (2005) showed that increasing dietary LA content in the diets significantly improved AA levels in chicken meat.

As we can see in Table 4 and Table 5, DPA was the major n-3 LCPUFA in breast and thigh. This may reflect the capacity for DPA to accumulate more readily in these tissues, with less being converted to DHA.

The greater DPA deposition achieved in breast and thigh samples compared to DHA may be due to the conversion of ALA to DPA following simple zero-order kinetics (Calder, 2016). Meanwhile, the synthesis of DHA from DPA is more complex in steps (Blank *et al*. 2002). Synthesis of ALA to DPA requires only one delta-6 desaturase pathway, whereas conversion from DPA to DHA requires a second pass after being elongated to 24:5n-3 (Calder, 2016). This results in competition between 24:5n-3 and LA in the use of delta-6 desaturase, which may reflect the complex kinetics between dietary ALA and DHA levels in tissue (Blank *et al*. 2002). In addition, 24:6n-3 needs to be beta-oxidized to DHA in peroxisomes, which provides another potential regulatory point. This indicates that the ratio of LA to ALA in diets is an important determinant for the optimal conversion of ALA to n-3 LCPUFA.

The level of thigh EPA and DHA of chickens fed dietary treatments with the highest LA to ALA ratio (Diet 3) decreased to 32.1 and 19.8% respectively compared to chickens fed the lowest LA to ALA ratio (Diet 1). The total n-3 LCPUFA (EPA, DPA, and DHA) levels in the breast and thigh of birds fed with the lowest LA content (2.9%en) was 15.6 and 18.7% (respectively) higher than the n-3 LCPUFA

in the breast and thigh of birds fed the highest LA content or the highest LA to ALA ratio (4.4%en). These findings indicate that the LA content in the diets is important in regulating the n-3 LCPUFA accumulation in tissues. This suggests that the decrease in n-3 LCPUFA levels might be due to competition between ALA, the precursor to n-3 LCPUFA, and LA for delta-6 desaturase (Husted and Bouzinova, 2016). Thus, a high dietary level of LA might depress the conversion of ALA to n-3 LCPUFA.

The result in the current study is in accordance with a research carried out by Zelenka *et al*. (2008) which found that the increasing level of LA in chicken diet was associated with significantly increasing levels of all n-6 PUFA in chicken meat.

Conversely, while the increased levels of LA tended to decrease the level of total n-3 PUFA (P<0.01), the level of total n-6 fatty acids increased (P<0.01) in liver, breast, and thigh meat.

LA content of liver, breast and thigh tissues fed diets containing 4.4%en (the highest LA content) increased significantly by 15.3, 19.2, and 18.4%, respectively, compared to those fed diets containing 2.9%en (the lowest LA content) (P<0.05). The highest level of LA and total n-6 fatty acids in breast and thigh tissues was achieved at the highest level of dietary LA (P<0.01. In general, any increase in n-6 fatty acid level in breast and thigh tissues was offset by a decrease in the MUFA content of tissues (P<0.05).

The results clearly indicated that the fatty acid profiles of the diets and proportions determine the fatty acid composition in tissues including liver, breast, and thigh. As reviewed by Royan and Navidshad (2015), the composition of various bird tissues was determined by dietary fats and the fatty acid profile of chicken meat can simply be modified by feed.

Moreover, Kanakri *et al*. (2018) reported that there were strong correlations and regressions between diet and fatty acid levels in broiler tissues confirming the ability to predict the tissue fatty acid profiles based on their composition of dietary fats. However, by the increase in meat LA, n-6 LCPUFA, AA, did not respond. This indicated that the concentration of LA was not translated to changes in the levels of meat AA.

Table 4 Fatty acid profiles of breast phospholipids from chickens fed diets varying in linoleic acid (LA) to alpha-linolenic acid (ALA) for 28 days[1]

Item	Experimental Diets				
	T1	T2	T3		
LA (% en)	2.90	3.78	4.38		
ALA (% en)	2.12	2.06	2.13		
LA:ALA ratio	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.11	5.18	5.15		
Fatty acids	**(% of total fatty acids)**			**PSEM**	**Significance**
16:0	20.86	21.18	21.48	0.274	NS
18:0	9.78	9.49	9.93	0.246	NS
SFA	38.36^{a}	38.03^{a}	39.40^{b}	0.089	**
18:1n-9	17.75	16.94	15.07	0.856	NS
18:1n-7	5.05	4.59	3.84	0.210	NS
MUFA	26.51^{b}	25.24^{b}	21.56^{a}	0.750	*
20:3n-9	0.43^{b}	0.41^{b}	0.26^{a}	0.025	*
18:2n-6 (LA)	14.80^{a}	16.97^{b}	18.31^{c}	0.222	**
20:3n-6	1.27	1.52	1.45	0.083	NS
20:4n-6 (AA)	3.62	4.12	4.78	0.235	NS
Total n-6	21.63^{a}	24.98^{b}	27.28^{c}	0.438	**
18:3n-3 (ALA)	1.52	1.30	1.34	0.063	NS
20:3n-3	0.97	0.89	0.96	0.067	NS
20:5n-3 (EPA)	2.63^{b}	2.17^{a}	2.00^{a}	0.051	**
22:5n-3 (DPA)	4.24	3.76	4.11	0.244	NS
22:6n-3 (DHA)	3.23	2.73	2.63	0.117	NS
Total n-3	12.66^{b}	10.90^{a}	11.09^{a}	0.300	*
Total PUFA	34.29	35.89	38.37	0.691	NS
	Fat Content (% fresh weight)				
	0.84	0.85	0.87	0.939	NS

[1] Values are means of twelve observations (n=12) per treatment and their pooled standard error of the mean (PSEM).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid and PUFA: polyunsaturated fatty acid.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.

Table 5 Fatty acid profiles of thigh phospholipids from chickens fed diets varying in linoleic acid (LA) to alpha-linolenic acid (ALA) for 28 days[1]

Item	Experimental Diets				
	T1	T2	T3		
LA (% en)	2.90	3.78	4.38		
ALA (% en)	2.12	2.06	2.13		
LA:ALA ratio	1.37:1	1.83:1	2.06:1		
Fat Content (%)	5.11	5.18	5.15		
Fatty acids	**(% of total fatty acids)**			**PSEM**	**Significance**
16:0	20.16	21.11	21.17	0.337	NS
18:0	13.04	12.63	12.91	0.323	NS
SFA[2]	37.40^{a}	37.94^{ab}	38.52^{b}	0.081	**
18:1n-9	19.54	18.71	16.42	0.747	NS
18:1n-7	4.61^{b}	4.26^{b}	3.66^{a}	0.147	*
MUFA	27.32^{b}	25.95^{b}	22.27^{a}	0.660	*
20:3n-9	0.37	0.33	0.22	0.028	NS
18:2n-6 (LA)	16.67^{a}	18.47^{b}	20.42^{c}	0.324	**
20:3n-6	0.96	1.04	1.02	0.052	NS
20:4n-6 (AA)	4.27	4.66	5.37	0.193	NS
Total n-6	23.49^{a}	26.13^{b}	29.15^{c}	0.388	**
18:3n-3 (ALA)	1.48^{b}	1.17^{a}	1.25^{a}	0.028	*
20:3n-3	0.69	0.66	0.75	0.067	NS
20:5n-3 (EPA)	2.15^{b}	1.59^{a}	1.46^{a}	0.062	**
22:5n-3 (DPA)	4.12	3.71	3.93	0.160	NS
22:6n-3 (DHA)	2.63^{b}	2.18^{a}	2.11^{a}	0.092	*
Total n-3	11.10^{b}	9.35^{a}	9.53^{a}	0.259	*
Total PUFA	34.60^{a}	35.48^{a}	38.69^{b}	0.627	*
	Fat Content (% fresh weight)				
	1.07	0.91	1.14	0.368	NS

[1] Values are means of twelve observations (n=12) per treatment and their pooled standard error of the mean (PSEM).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid and PUFA: polyunsaturated fatty acid.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.

These findings also agreed with studies reported previously by Rett and Whelan (2011) which found that when dietary LA levels in adult human clinical trials were increased up to six fold, there was no significant correlations with the arachidonic acid levels in plasma phospholipids.

The increased levels of dietary LA from 2.9 to 4.4%en did not influence the meat fat content including breast (0.85%) and thigh (1.04%), and the final weight of the birds at 28 days of age. This result is in concordance with those of Zelenka *et al*. (2008) using increased levels of dietary LA. Their results show that there was no difference in the fat content of breast meat due to the increased levels of dietary LA from 14.22 to 58.44 g/kg. In addition, diets supplemented with different dietary fats, including beef tallow, corn, flaxseed, canola, or coconut oil up to a level of 4% (w/w) did not change the lipid content of different broiler tissues (Kanakri *et al*. 2018). The experimental diets did not significantly influence the final weight of birds with an average of 1510 g. A number of investigators (Febel *et al*. 2008; Kavouridou *et al*. 2008) have reported similar findings regarding the final weight among broilers fed different types of fat sources involving vegetable oils.

Future studies should be conducted using dietary approaches to increase the quality and production of n-3 LCPUFA in chicken products. One compound which has beneficial health effects and potential to provide consumers with a functional chicken meat is conjugated linoleic acid (CLA).

Conjugated linoleic acid is a specific isomer of linoleic acid, which could be supplemented into the diets of chickens. This compound in nature is produced as a by-product of the fatty acid biohydrogenation in the rumen (Nosrati *et al*. 2015). The use of CLA in chicken diets has been suggested to enhance the n-3 fatty acid content and decrease the ratio of n-6 to n-3 fatty acids in meat (Royan *et al*. 2015). In addition, the use of vitamin C in the diets might improve the levels of n-3 fatty acids in chicken meat. A study conducted by Tavakoli *et al*. (2020) reported that chicken diets supplemented with 200 mg/kg vitamin C reduced the amount of saturated fatty acid, such as myristic acid, palmitic acid and stearic acid, and increased the level of unsaturated fatty acids.

CONCLUSION

The present study confirms previous findings and contributes additional evidence that the fatty acid composition of the tissues (liver, breast, and thigh) reflects the fatty acid profiles of the chicken diets. The changes in dietary linoleic acid in chicken diets lead to changes in phospholipid fatty acid composition in liver, breast, and thigh chicken meat. A high dietary LA to ALA ratio produced a lower level of ALA, n-3 LCPUFA (specifically EPA and DHA), and total n-3 fats in broiler chicken meat and resulted in a greater level of LA and total n-6 PUFA concentrations. In contrast, a low dietary LA to ALA ratio resulted in chicken meat with a higher n-3 LCPUFA and total n-3 fats. We conclude that diets that are lower in LA content will allow greater conversion of ALA into n-3 LCPUFA in broiler chicken meat**.** This strategy could help to provide a variety of foods rich in n-3 LCPUFA that may have positive impacts on human health.

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REFERENCES

Ayerza R. (2009). The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica*) as affected by environmental factors. *J. Oleo Sci.* **58(7),** 347-354.

Blank C., Neumann M.A., Makrides M. and Gibson R.A. (2002). Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. *J. Lipid Res.* **43(9),** 1537-1543.

Calder P.C. (2016). Docosahexaenoic Acid. *Ann. Nutr. Metab.* **69(1),** 7-21.

El-Bahr S.M., Shousha S., Alfattah M.A., Al-Sultan S., Khattab W., Sabeq I.I., Ahmed-Farid O., El-Garh K.A., Albusadah K.A., Alhojaily S. and Shehab A. (2021). Enrichment of broiler chickens' meat with dietary linseed oil and lysine mixtures: Influence on nutritional value, carcass characteristics and oxidative stress biomarkers. *Foods*. **10(3),** 618-635.

Febel H., Mezes M., Palfy T., Herman A., Gundel J., Lugasi A., Balogh K., Kocsis I. and Blazovics A. (2008). Effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. *J. Anim. Physiol. Anim. Nutr.* **92(3),** 369-376.

Folch J., Lees M. and Sloane Stanley G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226,** 497-509.

Gibson R.A., Neumann M.A., Lien E.L., Boyd K.A. and u W.C. (2013). Docosahexaenoic acid synthesis from alpha-linolenic acid is inhibited by diets high in polyunsaturated fatty acids. *Prostaglandins Leukot. Essent. Fatty Acids.* **88(1),** 139-146.

Husted K.S. and Bouzinova E.V. (2016). The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. *Medicina*. **52(3),** 139-147.

Kalakuntla S., Nagireddy N.K., Panda A.K., Jatoth N., Thirunahari R. and Vangoor R.R. (2017). Effect of dietary incorporation of n-3 polyunsaturated fatty acids rich oil sources on fatty acid profile, keeping quality and sensory attributes of broiler chicken meat. *Anim. Nutr.* **3(4),** 386-391.

Kanakri K., Carragher J., Hughes R.J., Muhlhausler B. and Gibson R.A. (2018). The effect of different dietary fats on the fatty acid composition of several tissues in broiler chickens. *European J. Lipid Sci. Technol.* **120(1),** 1700237.

Kartikasari L.R., Geier M.S., Hughes R.J., Bastian S.E.P. and Gibson R.A. (2021). Omega-3 fatty acid levels and sensory quality of eggs following consumption of alpha-linolenic acid enriched diets. *Food Res.* **5(2),** 57-64.

Kartikasari L.R., Hughes R.J., Geier M.S., Makrides M. and Gibson R.A. (2012). Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. *Prostaglandins Leukot. Essent. Fatty Acids.* **87(4),** 103-109.

Kavouridou K., Barroeta A.C. and Villaverde C. (2008). Fatty acid, protein and energy gain of broilers fed different dietary vegetable oils. *Spanish J. Agric. Res*. **6,** 210-218.

Komprda T., Zelenka J., Fajmonova E., Fialova M. and Kladroba D. (2005). Arachidonic acid and long-chain n-3 polyunsaturated fatty acid contents in meat of selected poultry and fish species in relation to dietary fat sources. J. Agric. Food Chem. **53,** 6804-6812.

Liou Y.A., King D.J., Zibrik D. and Innis S.M. (2007). Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J. Nutr.* **137(4),** 945-952.

Nain S., Renema R.A., Korver D.R. and Zuidhof M.J. (2012). Characterization of the n-3 polyunsaturated fatty acid enrichment in laying hens fed an extruded flax enrichment source. *Poult. Sci.* **91(7),** 1720-1732.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Nguyen D.V., Malau-Aduli B.S., Cavalieri J., Nichols P.D. and Malau-Aduli A.E.O. (2018). Supplementation with plant-derived oils rich in omega-3 polyunsaturated fatty acids for lamb production. *Vet. Anim. Sci.* **6,** 29-40.

Nosrati M., Navidshad B. and Malecky M. (2015). Effects of dietary conjugated linoleic acid and n-3 fatty acids on the performance, carcass traits and small intestinal morphology of broiler chickens. *Iranian J. Appl. Anim. Sci.* **5(2),** 411-416.

Rett B.S. and Whelan J. (2011). Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults

consuming Western-type diets: a systematic review. *Nutr. Metab.* **8(36),** 1-15.

Rodríguez R., Rebollar P.G., Mattioli S. and Castellini C. (2019). n-3 PUFA sources (precursor/products): A review of current knowledge on rabbit. *Animals.* **9(806),** 1-17.

Royan M. and Navidshad B. (2015). The metabolic effects of conjugated linoleic acids (CLA) in chickens: A review. *Iranian J. Appl. Anim. Sci.* **5(3),** 517-528.

Royan M., Navidshad B. and Akhlaghi A. (2015). The role of dietary fat to produce chicken meat as a functional food: A review. *Iranian J. Appl. Anim. Sci.* **5(1),** 5-12.

Simopoulos A.P. (2016). An Increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*. **8(128),** 1-17.

Stanacev V.Z., Milosevic N., Pavlovski Z., Millic D., Vukic-Vranjes M. and Puvača N. (2014). Effects of dietary soybean, flaxseed and rapeseed oil addition on broilers meat quality. *Biotechnol. Anim. Husb.* **30(4),** 677-685.

Tavakoli M., Bouyeh M. and Seidavi A.R. (2020). Effects of dietary vitamin C supplementation on fatty acid profile in breast meat of broiler chickens. *Meso*. **22(4),** 268-273.

Tocher D.R., Betancor M.N., Sprague M., Olsen R.E. and Napier J.A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. *Nutrients*. **11(89),** 1-20.

Tu W.C., Cook-Johnson R.J., James M.J., Muhlhausler B.S. and Gibson R.A. (2010). Omega-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression. *Prostaglandins Leukot. Essent. Fatty Acids*. **83,** 61-68.

Uddin M.K., Juraimi A.S., Hossain M.S., Nahar M.A.U., Ali M.E. and Rahman M.M. (2014). Purslane weed (*Portulaca oleracea*): A prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. *Sci. World J.* **2014,** 1-6.

Zelenka J., Schneiderova D., Mrkvicova E. and Dolezal P. (2008). The effect of dietary linseed oils with different fatty acid pattern on the content of fatty acids in chicken meat. *Vet. Med*. **53(2),** 77-85.